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Investigation of travel-related cases in a multinational outbreak: example of the Shiga-toxin producing *E. coli* outbreak in Germany, May–June 2011

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SUMMARY

Early investigation of travel-related cases in an outbreak of an emerging infectious disease can provide useful information to epidemiologists to characterize the exposure, while they may differ in demographic profiles from cases reported in the country where the outbreak has occurred. During the spring 2011 *E. coli* outbreak in Germany, we proposed a methodological approach to collect a minimal set of demographic and clinical data that are relatively easy to obtain and available at an early stage of an outbreak investigation. Ninety-eight STEC O104 travel-related cases were reported in a survey by seven EU countries, Switzerland, Canada and the USA. We found a mean incubation period ($n = 50$) of 8·5 days, which confirmed previous estimations communicated by the Robert Koch Institute. No significant association was found between the duration of the incubation period and possible demographic and clinical factors, although the older the age, the shorter the incubation period that was observed. Such approach and observations are informative for further investigations of outbreaks of enterohaemorrhagic *E. coli* or other emerging infectious diseases.

Key words: *Escherichia coli* (*E. coli*), investigation, outbreaks, travellers' infection.

INTRODUCTION

In an international context, the monitoring of outbreak-related cases of infectious diseases who have travelled to a common affected area can provide useful information to identify the time and place of the exposure to a common source. This paper focuses on the example of the large outbreak of Shiga toxin-producing *Escherichia coli* (STEC) O104 that occurred in Germany from May to August 2011. By 16 August 2011, 3842 cases had been reported nationally to the Robert Koch Institute (RKI), including 2987 STEC cases and 855 haemolytic uraemic syndrome (HUS) cases. Fifty-three patients died, 35 of them from HUS [1–6]. On 22 May 2011, this outbreak was reported through the European Union (EU) Early Warning and Response System (EWRS), which is a confidential platform allowing EU Member States to send alerts about events with a potential impact on the EU, share information, and coordinate their response. The European Centre for Disease Prevention and Control (ECDC) [7] initiated a series of actions to support the investigations conducted in Germany [3–6]. An EU outbreak case definition for travel-related cases was developed on 7 June 2011 [8], harmonized with the case definition for HUS cases that was previously established by RKI. Daily epidemiological

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updates were published from 25 May until 27 July 2011, and a risk assessment first published on 27 May, was regularly updated by ECDC [9].

The objectives of the study were to describe a demographic, clinical and epidemiological profile of STEC O104:H4 epidemic cases with travel history to Germany, and to determine the incubation period and potential associated demographic and clinical factors of these travel-related cases. Importance of international collaboration and networking of experts is discussed in the light of needs for focused and accurate epidemiological studies utilizing precise information on exposure [10, 11].

METHODS

Data sources

Questionnaire and reporting system

ECDC and the International Health Regulation (IHR) focal points of affected countries, posted a questionnaire and a study protocol on 16 June 2011 via the EWRS. This questionnaire and a study protocol were developed to document potential dates and places of exposure for cases meeting the travel-related case definition. The variables collected were age, sex, country of notification and clinical presentation of the disease (diarrhoea, bloody diarrhoea, HUS) of STEC O104 cases. Date of onset of diarrhoea was used in the analysis.

For each individual case reported outside Germany with previous stay in Germany, travel activities were listed by date of arrival in, and date of departure from Germany as well as for each specific location (town, city) and place visited (e.g. restaurant, hotel) in chronological order. For each place visited, sprout consumption was queried as it was hypothesized as the vehicle of infection at the time of the study.

Data were submitted by EWRS national focal points from Denmark, France, Luxembourg, The Netherlands, Spain, Sweden, and the UK (Scotland) to ECDC. Data were also submitted by the IHR focal points from Canada, USA and Switzerland through the World Health Organisation (WHO-Euro). Data collection did not require re-interviewing cases, but was based on information already collected in each Member State.

Case definition

We included in the study cases of illness reported from 2 May 2011 until 30 June 2011 and meeting the case definition for probable or confirmed cases [8].

A confirmed case of STEC O104 was defined as any person meeting the criteria for a possible case, and isolation of a STEC strain of serotype O104:H4, or isolation of a STEC strain of serotype O104 and fulfilling epidemiological criteria for a probable case. A probable case of STEC O104 was defined as any person meeting the criteria for a possible case of STEC diarrhoea or STEC HUS and, during the 14-day exposure period before the onset of illness, meeting at least one of the following epidemiological criteria:

- stay in Germany or any other country where a confirmed case had probably acquired infection, but not usually living in Germany;
- consumption of food product obtained from Germany;
- close contact (e.g. in a household) with a confirmed epidemic case who had travelled to Germany during the outbreak period.

A possible case of STEC O104 was defined as occurring in a person who developed on or after 1 May 2011, STEC diarrhoea [defined as acute onset of diarrhoea or bloody diarrhoea and at least one of the following laboratory criteria: (1) isolation of an *E. coli* strain that produces Shiga toxin 2 (Stx2) or harbours the *stx2* gene; (2) direct detection of *stx2* gene nucleic acid in faeces without strain isolation] *or* STEC HUS (defined as haemolytic uraemic syndrome (HUS) defined as acute renal failure and at least one of the following clinical criteria: microangiopathic haemolytic anaemia, thrombocytopenia].

The German case definition of Shiga-toxin-producing *E. coli* gastroenteritis (without HUS) required, besides laboratory confirmation, the presence of at least one of the following symptoms: diarrhoea ($\geqslant 3$ loose stools in a 24-h period), abdominal cramps, or vomiting. In addition, physicians were required to report clinical symptoms compatible with diarrhoea-associated HUS in patients [2].

Data management and analysis

Data were analysed in terms of person, time and place using the statistical program Stata v. 10 (StataCorp., USA). We verified whether the percentage of HUS STEC cases reported in this study was different form the percentage of cases reported in the ECDC daily update since 30 June 2011. Country-specific median age and overall median age of cases were calculated with respective interquartile ranges (IQR). Sex and age distribution of cases were displayed for all countries, by 10-year age groups.

For all STEC cases, travel history was reconstructed by time and place (city, county, restaurant) visited during their journey.

The incubation period for a case was defined as the number of days between date of diarrhoea onset and probable exposure date. For the purpose of this analysis we selected the cases that stayed for ⩽3 days (72 h) in Germany, and individual incubation time was calculated from the mean date of the stay for each case. An incubation period was estimated as the median of all individual incubation times. Association between incubation period and quantitative variables (age, duration of stay) was tested by univariate regression analysis. Mean incubation periods were compared by categories of dichotomic variables (sex, HUS, hospitalization) using t tests.

RESULTS

Cases reported

Between 2 May and 30 June 2011, 98 travel-related confirmed and probable cases of STEC O104 were included in this study; 87 cases reported by EU/EEA countries and 11 cases reported by Canada, Switzerland and the USA (Table 1). Cases included from the EU/EEA countries (87 cases) accounted for 89% of the number of cases reported.

The percentage of HUS STEC cases reported in the ECDC Epi summary of 30 June for all cases (http://www.ecdc.europa.eu/en/healthtopics/escherichia_coli/Pages/index.aspx) was 35% and did not significantly differ from 38% reported in travellers in the study (χ^2, $P = 0{\cdot}73$).

Seventy-one percent of cases were reported by Sweden ($n = 48$) and Denmark ($n = 22$). Most of the cases reported by Sweden travelled to Germany as part of a group participating in a golf trip [12]. Eight cases reported by Denmark belonged to five independent groups of travellers: they visited the same restaurant in the Landkreis Schleswig-Flensburg where they are assumed to have become infected. Four other cases became infected during a golf holiday at the same resort as the group of Swedish cases. Furthermore, seven of the cases reported by Denmark had eaten at the same motorway rest stop near Hamburg on four different days [13].

The sex ratio (female:male) of STEC cases reported in this study was 1·2:1. Of the reported HUS cases, the sex ratio (female:male) was 2·8:1. The median age was 59 years (IQR 47–68 years). The median age varied by country of travellers' origin; from 39 years in cases reported by Spain to 68 years in those from Canada. Thirty-five percent of cases were aged between 60 and 69 years. Information on hospitalization of cases was available for 74 cases and their proportion of hospitalization was 64%. All cases reported with HUS were reported as having been hospitalized.

Date of onset and probable date of infection

The date of onset of symptoms (onset of diarrhoea) was reported for 71 STEC cases (HUS and non-HUS cases). The earliest date of onset for travel-related cases reported outside Germany was 12 May 2011. This case was reported by Luxembourg. In the curve presented in Figure 1, the dates with the highest number of cases are 16 May (nine cases), 18 May (seven cases) and 21 May (eight cases). The third peak in time corresponds to the peak of the outbreak reported in Germany. A large proportion of HUS STEC cases (82%) reported outside Germany had a date of onset before 24 May. The latest date of onset for a travel-related STEC case was 11 June for a case reported by the US CDC. The duration of travel in Germany ranged from 1 (<24 h) to 39 days. Out of 87 travel-related STEC cases, 58 (67%) stayed ⩽3 days (Fig. 2).

Incubation period

Information on date of onset was available for 50 of the 58 cases that stayed in Germany for ⩽3 days. The median incubation period for these 50 cases was 8 days with values distributed between 1·5 days to 20·5 days [10% of extreme estimated values (six values) are below 4 days or above 17 days]. The association of the incubation period and characteristics of cases is displayed in Table 2.

The mean incubation period was the highest in the 50–59 years age group and decreased in the older age groups and was highest for individuals who stayed 2 days.

The mean incubation period was not found to be different between gender, HUS status and hospitalization using a significance level of 0·05

DISCUSSION

The completeness of reporting for this investigation was good (79%). The proportion of HUS STEC

Table 1. *Number of probable and confirmed HUS and non-HUS STEC cases reported outside Germany, as of 30 June 2011*

	Number of STEC cases with travel history to Germany (ECDC Epi summary, 30 June 2011)			Number of STEC cases reported to the study, 30 June 2011			
	HUS	Non-HUS	Total	HUS	Non-HUS	Total	Median age, years (IQR)
Austria	1	4	5	–	–	–	–
Czech Republic	0	1	1	–	–	–	–
Denmark	9*	14	23	8	14	22	61 (49–64)
France	0	2	2	–	1	1	
Greece	0	1	1	–	–	–	–
Luxembourg	1	1	2	1	1	2	42 (33–51)
The Netherlands	4	7	11	4	7	11	48 (27–68)
Norway	0	1	1*	0	–	–	–
Poland	2	1	3	–	–	–	–
Spain	1	1	2	1	1	2	39·5 (36–43)
Sweden	18	35	53	18	30	48	63 (53–68)
UK	3	3	6	1	0	1	61
Canada	–	–	–	0	1	1	68
Switzerland	–	–	–	0	5	5	40 (38–40)
USA	–	–	–	4	1	5	52 (42–53)
Total	39	71	110	37	61	98	59 (47–68)

HUS, Haemolytic uraemic syndrome; STEC, Shiga toxin-producing *Escherichia coli*; IQR, interquartile range.
* One case from Denmark and one case from Norway did not have any travel history to Germany. They were excluded from the study.

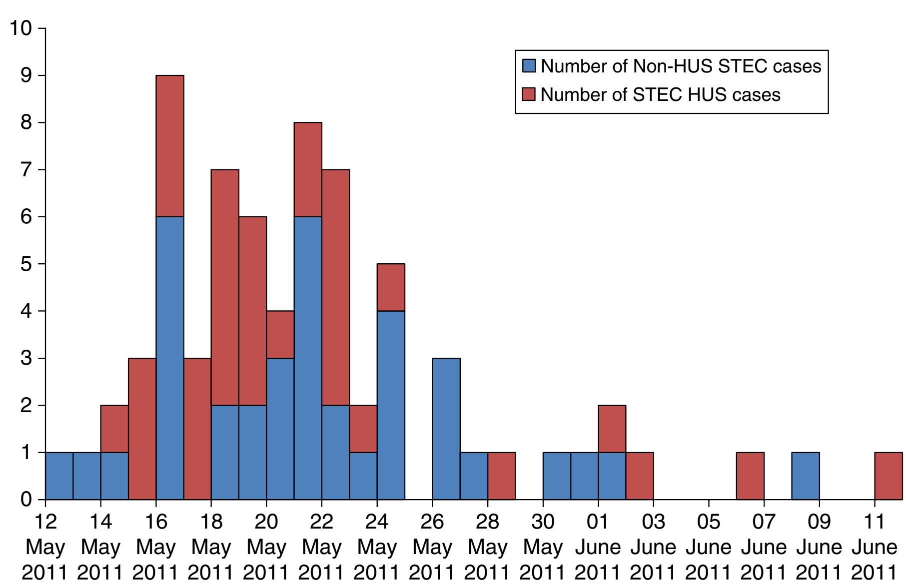

Fig. 1. Distribution of probable and confirmed travel-related HUS and non-HUS STEC O104 cases reported outside Germany by date of onset ($n = 71$), 1 May–30 June 2011.

cases reported in this study (38%) is higher than that reported in Germany (25%) [2]. This could be explained by the heightened attention for uncomplicated STEC diarrhoea during the outbreak monitoring in Germany, and by the fact that diarrhoea cases may have occurred in other countries but were not diagnosed or reported to the public health system.

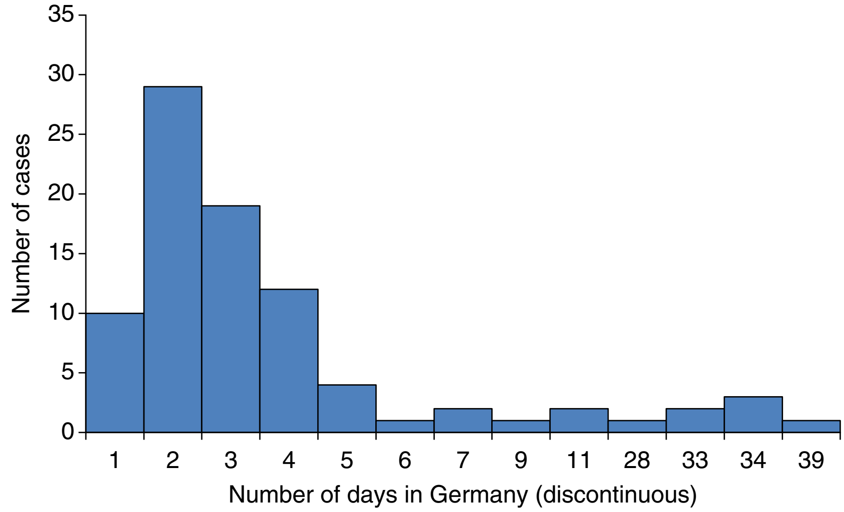

Fig. 2. Distribution of travel-related STEC O104 cases reported outside Germany by length of stay in Germany ($n = 87$), 1 May–30 June 2011.

Table 2. *Association of incubation period and characteristics of travel-related STEC cases (N = 50)*

Variable	Mean incubation period, days (IQR)	Result
Age, years		$r = -0{\cdot}52$, $P > 0{\cdot}05$
30–39 ($n = 5$)	8·9 (4·4–13·4)	
40–49 ($n = 11$)	7·3 (5·1–9·4)	
50–59 ($n = 8$)	9·8 (6·7–12·9)	
60–69 ($n = 17$)	8·4 (6·9–9·9)	
70–79 ($n = 8$)	8·5 (4·0–13·0)	
Duration of stay		$r = -0{\cdot}45$, $P > 0{\cdot}05$
1 day ($n = 9$)	8·0 (5·4–10·7)	
2 days ($n = 22$)	9·3 (7·8–10·8)	
3 days ($n = 19$)	8·0 (6·0–10·0)	
Sex		$t = 0{\cdot}79$, $P > 0{\cdot}05$
Female ($n = 27$)	8·9 (7·6–10·2)	
Male ($n = 21$)	8·0 (6·1–9·9)	
HUS status		$t = -0{\cdot}3$, $P > 0{\cdot}05$
Non-HUS ($n = 21$)	8·5 (6·8–10·3)	
HUS ($n = 21$)	8·9 (7·4–10·3)	
Hospitalization		$t = -1{\cdot}2$, $P > 0{\cdot}05$
Not hospitalized ($n = 13$)	6·9 (5·1–8·7)	
Hospitalized ($n = 24$)	8·2 (7·0–9·4)	

R, Correlation coefficient; *t, t* test for mean.

We verified that all HUS STEC cases were hospitalized. The median age of cases reported in this study is higher than that reported in Germany for all cases identified (60·5 *vs.* 47 years). This can probably be explained by the profiles of travellers, as some were part of organized tours with groups of older people.

The epidemic curve did not continue to increase sharply after 16 May, in contrast to the trend reported for STEC cases in Germany [2]. This could be explained by several different hypotheses. For example, the tour operators could have changed programmes if they were informed that travellers on the last trip became ill (without necessarily knowing about the outbreak).

The median incubation period estimated (8·5 days) in this study corresponds with results already reported elsewhere for this particular strain of STEC O104, being 8 days [2, 13]. However, this estimated incubation period is unexpectedly high compared to the reported incubation period for EHEC/STEC O157, being around 3–4 days [14]. Information on the incubation period can be useful not only to identify the source of contamination, but also to target preventive measures for persons at risk of developing an infection (because of a common exposure) or having contact with an index case. For example, in the case of the STEC outbreak in Germany, questions could be asked about various food exposures during the 4–17 days (90% of the values were between 4 and 17 days) preceding the date of onset, so that a vehicle of infection could be investigated. The dispersion of this estimated incubation period is large, but, the outliers may not have been confirmed STEC O104 cases as the information on case classification was not available for all cases. Results of demographic and clinical factor analysis did not show any significant association between the incubation period and characteristics of cases, although a negative association was found with age as verified in a later study [15]. Retrospective analysis of demographic and clinical factors associated with incubation period in the context of foodborne- and waterborne-related outbreaks, could be useful to better characterize the host–pathogen dynamic.

In this study, it was not possible to collect information on the potential vehicle of infection (sprouts), due to recall bias. Sprouts are often used as a decoration of dishes and sandwiches; consumers are not always aware of their presence. The common vehicle of infection was found through a recipe-based restaurant cohort study, conducted by RKI [10, 11]. A scientific opinion on the risk posed by STEC and other pathogenic bacteria in seeds and sprouted seeds was published by the European Food and Safety Agency [16, 17]. Sprout-associated outbreaks as well as other food-related outbreaks can have an international dimension due to the global food trade. Early investigation of travel-associated cases in the context of food-related outbreaks can be a useful indication of potential places of infection and help target field investigations aimed at identification of the source of contamination. Furthermore, a retrospective cohort analysis of travel-related cases can provide more specific indication on exposure to contaminated food items if conducted on time [12, 13].

CONCLUSION

A coordinated approach was developed to collect information and conduct an early investigation of travel-related STEC cases including outliers (unusual incubation period). Early investigation of travel-associated cases can prove to be extremely useful, as the investigation of the exposure allows a greater focus on time and place, especially when journeys are short and at a specific location. Such information is difficult to obtain from investigations of autochthonous cases. The data collected were complementary to the data from the non-travel-related cases reported to RKI at the time of the outbreak. Moreover, groups of exposure were identified through the travel-related cases, which have been further investigated by the public health services of the respective countries.

We tested and verified the hypothesis, revealed in the preliminary findings, that the incubation period for the strain responsible of this outbreak was longer than the average (i.e. 3–4 days) as reflected in the literature for previously known STEC strains. We also verified that there was no significant association between the incubation period and the characteristics of cases (age, sex, duration of stay, HUS status, hospitalization). When a large foodborne outbreak occurs in a European country, collecting information on travel-related cases at the European/international level at an early stage can include demographic (age, gender, country of origin), and geographical (probable places of exposure) information and clinical parameters (symptoms, severity). This is especially relevant when a new strain of a pathogen is involved [18]. Such a method can be used as a case-study that can be applied to other foodborne and waterborne outbreaks with a potential international dimension.

ECDC has supported the early collection of information on travel-related cases through appropriate channels (EWRS), developed a common European case definition and platform for the joint analysis of data, exchange of information between countries and risk assessment at the European level. To ensure an efficient support for such an outbreak investigation at international level, formulation of hypothesis and coordination of appropriate response (including selection and implementation of appropriate protocols for epidemiological investigations) must be conducted in a timely manner between epidemiologists at local, national and international levels.

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DECLARATION OF INTEREST

None.

REFERENCES

1. **Robert Koch Institute.** Report: Final presentation and evaluation of epidemiological findings in the EHEC O104:H4 outbreak, Germany 2011. Berlin, 2011.
2. **Frank C, *et al.* for the HUS Investigation Team.** Epidemic profile of shiga-toxin-producing *Escherichia coli* O104:H4 outbreak in Germany. *New England Journal of Medicine* 2011; **365**: 1771–1780.
3. **Frank C, *et al.*, on behalf of the HUS Investigation Team.** Large and ongoing outbreak of haemolytic uraemic syndrome, Germany, May 2011. *Eurosurveillance* 2011; **16**(21): pii=19878.
4. **Askar M, *et al.*** Update on the ongoing outbreak of haemolytic uraemic syndrome due to Shiga toxin-producing Escherichia coli (STEC) serotype O104, Germany, May 2011. *Eurosurveillance* 2011; **16**(22): pii=19883.
5. **Wadl M, *et al.*, on behalf of the HUS Surveillance and Laboratory Team.** Enhanced surveillance during a large outbreak of bloody diarrhoea and haemolytic uraemic syndrome caused by Shiga toxin/verotoxin-producing Escherichia coli in Germany, May to June 2011. *Eurosurveillance* 2011; **16**(24): pii=19893.
6. **Altmann M, *et al.*** Timeliness of surveillance during outbreak of Shiga toxin-producing Escherichia coli, Germany, 2011. *Emerging Infectious Diseases* 2011; **17**: 1906–1909.
7. **European Parliament.** Regulation (EC) No. 851/2004 of the European Parliament and of the Council of 21 April 2004 establishing a European Centre for disease prevention and control (http://eur-lex.europa.eu/legal-content/EN/ALL/;ELX_SESSIONID=zVFMJ9lLPSp4QZbGs6mhn FH0CWGlSnngYG9cn1xJsfQ1Yz2ZpXc4!-13 23427427?uri=CELEX:32004R0851).
8. **European Centre for Disease Control and Prevention.** EU case definition for diarrhoea and haemolytic uremic syndrome (HUS) caused by the epidemic strain Shiga toxin 2-producing *Escherichia coli* (STEC) O104:H4 (http://ecdc.europa.eu/en/healthtopics/escherichia_coli/epidemiological_data/Pages/EU_case_definition.aspx).
9. **ECDC Rapid Risk Assessment.** Outbreak of Shiga toxin-producing *E. coli* (STEC) in Germany, 27 May 2011. (http://ecdc.europa.eu/en/publications/Publications/1105_TER_Risk_assessment_EColi.pdf).
10. **Buchholz U, *et al.*** German outbreak of *Escherichia coli* O104:H4 associated with sprouts. *New England Journal of Medicine* 2011; **365**: 1763–1770.
11. **The Federal Institute for Risk Assessment (BfR), Federal Office of Consumer Protection and Food Safety (BVL) and Robert Koch Institute (RKI).** EHEC: current state of knowledge concerning illnesses in humans. Joint Declaration, 10 June 2011 (http://www.bfr.bund.de/en/press_information/2011/16/ehec__current_state_of_knowledge_concerning_illnesses_in_humans-70978.html).
12. **Ivarsson S, *et al.* and Regional Investigation Team.** Rapid detection and communication of Swedish cases provided early puzzle pieces in the German STEC O104:H4 outbreak. Poster presented in ESCAIDE, 6–8 November 2011.
13. **Müller L, *et al.*** Danish outbreak investigation related to a large outbreak of shiga toxin-producing *E. coli* O104 in Germany, May-June 2011 – the important role of outlier investigation ESCAIDE, Stockholm, November 2011.
14. **Reijn E, *et al.*** Analysis of timeliness of infectious disease reporting in the Netherlands. *BMC Public Health* 2011, **11**: 409.
15. **Werber D, King LA, Müller L.** Associations of age and sex with the clinical outcome and incubation period of shiga toxin-producing Escherichia coli O104:H4 infections, 2011. *American Journal of Epidemiology* 2013; **178**: 984–992.
16. **EFSA Panel on Biological Hazards (BIOHAZ).** Scientific Opinion on the risk posed by Shiga toxin-producing *Escherichia coli* (STEC) and other pathogenic bacteria in seeds and sprouted seeds. *EFSA Journal* 2011; **9**(11): 2424 (101 pp.).
17. **European Food Safety Authority.** Urgent advice on the public health risk of Shiga-toxin producing *Escherichia coli* in fresh vegetables. *EFSA Journal* 2011; **9**(6): 2274 (50 pp.).
18. **Goodman RA, Buehler JW, Koplan JP.** The epidemiologic field investigation: science and judgement in public health practice. *American Journal of Epidemiology* 1990; **132**: 9–16.